AD_____

Award Number: DAMD17-02-1-0502

TITLE: Screening for ATM Mutations in an African American Population to Identify a Predictor of Breast Cancer Susceptibility

PRINCIPAL INVESTIGATOR: Barry S. Rosenstein, Ph.D.

CONTRACTING ORGANIZATION: Mount Sinai School of Medicine
New York, NY 10029-6574

REPORT DATE: July 2005

20060309 111

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE *(DD-MM-YYYY)* 01-07-2005	2. REPORT TYPE Annual	3. DATES COVERED *(From - To)* 1 Jul 2004 – 30 Jun 2005

4. TITLE AND SUBTITLE
Screening for ATM Mutations in an African American Population to Identify a Predictor of Breast Cancer Susceptibility

5a. CONTRACT NUMBER

5b. GRANT NUMBER
DAMD17-02-1-0502

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Barry S. Rosenstein, Ph.D.

E-Mail: barry.rosenstein@mssm.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Mount Sinai School of Medicine
New York, NY 10029-6574

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The hypothesis being tested in this project is that a greater proportion of African-Americans with breast cancer harbor a specific germline genetic alteration in the *ATM* gene or possess a particular *ATM* haplotype, compared to African-American women without breast cancer. An additional objective is to determine the functional impact upon the protein encoded by the *ATM* gene for each mutation identified.
Specific Aims: The specific aims of this project are to (1) screen 100 African-American breast cancer patients and 100 African-American women without breast cancer and (2) perform functional studies using cells from patients identified as *ATM* carriers to determine whether each *ATM* genetic variant identified affects radiosensitivity and levels of the protein encoded by the *ATM* gene for each mutation examined.
The main accomplishments during this past year were to perform functional assays with a series of patient derived cell lines to measure radiosensitivity, ATM protein levels and phosphorylation of p53 by ATM.

15. SUBJECT TERMS
No abstract provided.

16. SECURITY CLASSIFICATION OF:

a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 11	19a. NAME OF RESPONSIBLE PERSON

19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction

African-American women on average present with more advanced breast cancer when compared with Caucasian women. This leads to suboptimal cure rates within this population. Numerous investigators have attempted to explain this discrepancy and determine if it stems from an inherent aggressive biologic behavior or a lack of access to appropriate medical care. It is still controversial, but there is evidence from randomized trials that when controlled for stage, African-American women have similar outcomes compared with their ethnic counterparts. However, it appears that socioeconomic factors lead to delayed screening evaluations and disease that is locally or systemically advanced rather than pre-emptive identification of early stage breast cancer. It would therefore be useful to discover a genetic marker that can serve to identify African-American women who are at increased risk for breast cancer at an age prior to disease development. The *ATM* gene has been chosen for consideration as a potential marker given its critical role in the maintenance of genomic integrity.

The hypothesis to be tested in this project is that a greater proportion of African-Americans with breast cancer harbor a genetic alteration in the *ATM* gene compared to

African-American women without breast cancer. An additional objective is to determine the functional impact upon the protein encoded by the *ATM* gene for each mutation identified.

The specific aims of this project are to (1) screen 100 African-American breast cancer patients and 100 African-American women without breast cancer and (2) perform functional studies using cells from patients identified as *ATM* carriers to determine whether each *ATM* variant identified affects radiosensitivity and levels of the protein encoded by the *ATM* gene for each mutation identified.

To accomplish this work, blood lymphocytes will be isolated from African-American breast cancer patients as well as non-breast cancer controls. DNA is isolated from these cells and each of the coding exons of the *ATM* gene for every patient will be screened for *ATM* alterations using denaturing high performance liquid chromatography (DHPLC). In those exons that display aberrant DHPLC profiles suggestive of a mutation, DNA sequencing will be performed to identify and characterize the mutation. For people diagnosed as *ATM* carriers, lymphoblastoid cell lines will be created to analyze ATM protein functional status.

Body

Due to the substantial delay until April 30, 2003 from the HSRRB (Human Subjects Review Board) of the DOD for approval of the human subjects protocol and consent forms for this project, which was followed by an additional delay to obtain approval from both the Mount Sinai and NYU IRBs, subject accrual into this study could not be initiated until the beginning of the second year of this project. Because of this delay, the U.S. Army Medical Research Acquisition Activity was notified on June 1, 2005 that we will exercise the option for a 12-month no-cost extension of this grant to enable completion of the work in this project. This will result in a revised termination date for this project of June 30, 2006. This was done per the Assistance Agreement, section 4.C. - "The recipient may make a one-time "no-cost" extension to the expiration date of the award for a period up to 12 months. The recipient shall notify the grants officer, in writing, at least 10 days prior to the expiration date of the award."

Since much of the genetic screening was accomplished in the previous year, most of the work in the past year focused upon performance of functional assays to determine the effect of *ATM* sequence variants on the function of the ATM protein using lymphoblastoid cell lines derived from EBV transformed lymphocytes obtained from subjects all of whom were never diagnosed with breast cancer. This work was performed using 5 cell lines from subjects who were not found to possess an *ATM* variant and 12 subjects who were identified with *ATM* variants. It should be noted that radiosensitivity was measured in each cell line. However, the experiments were not completed to measure ATM and p53 phosphorylation in all cell lines. The designation NC (not complete) is indicated in these cases.

4

For experiments in which p53 phosphorylation was measured, cells were irradiated with either 0 or 4 Gy of x-rays and incubated either 0.5 or 2 hr. The densitometric results for each time point were divided by the value in each experiment for unirradiated cells to normalize these results. Each irradiation was performed a total of three times. The mean values (with standard deviations) for wild type cells incubated either 0.5 or 2.0 hr were 2.6+2.0 or 3.8+2.5, respectively. The results for the cell lines possessing variants are shown in Table 1. In addition, ATM protein levels were measured in each cell line irradiated in three separate experiments and divided by the average value obtained for the five wild type *ATM* cell lines.

Table 1. Functional Assays of Lymphoblastoid Cells Derived from Subjects Possessing *ATM* Variants

Cell Line	Nucleotide	Change	Amino Acid Position	Substitution	ATM level	phospho p53 0.5 hr	phospho p53 2 hr
MS01-83	378	T>A	126	D>E	1.13+0.49	1.06+0.13	1.79+0.75
	1176	C>G	392	G>G			
MS02-27	378	T>A	126	D>E	0.97+0.35	2.09+0.84	1.57+0.84
	1254	A>G	418	Q>Q			
	2289	T>A	763	F>L			
MS02-36	3383	A>G	1128	Q>R	NC	NC	NC
MS02-47	1541	G>A	514	G>D	0.87+0.26	NC	NC
	4138	C>T	1380	H>Y			
MS02-57	2614	C>T	872	P>S	1.58+0.39	3.28+3.60	2.34+0.80
	2685	A>G	895	L>L			
MS02-70	378	T>A	126	D>E	NC	NC	NC
	6437	G>C	2146	S>T			
	9215	A>G	n/a	n/a			
MS02-71	378	T>A	126	D>E	NC	2.21+2.56	4.98+4.55
	2096	A>G	699	E>G			
MS03-06	1073	A>G	358	N>S	NC	NC	NC
	2362	A>C	788	S>R			
MS03-10	378	T>A	126	D>E	1.08+0.38	NC	NC
	6176	C>T	2059	T>I			

MS03-38	1254	A>G	418	Q>Q	NC	NC	NC
	1541	G>A	514	G>D			
MS03-39	975	A>C	1992	K>T	0.72+0.29	1.58+1.63	3.02+3.15
	IVS62+8	A>C	n/a				
MS03-46	378	T>A	126	D>E	0.50+0.23	1.18+0.25	2.40+1.54
	544	G>C	182	V>C			

The only instance in which a statistically significant result was obtained was for MS03-46, which exhibited a significantly lower level of ATM protein. In addition, the cell line derived from this individual had a lower level of p53 phosphorylation. This woman possessed both 378T>A and 544G>C variants. It would appear unlikely that the 378T>A was responsible for the diminished ATM response as this SNP was present in several other subjects that showed normal ATM levels and p53 phosphorylation. Possibly the 544G>C variant impacts ATM and this will be tested through analysis of other cell lines derived from patients with this genetic alteration.

The radiosensitivity of each cell line was also determined from the growth response of cells irradiated with either 0, 0.5, 1.0 or 2.0 Gy of X-rays by extrapolating the growth curves to the intercept at zero time. One example is shown for wild type cells irradiated with 4 Gy. The dose response based upon each growth curve is then provided. Three cell lines MS03-39, MS02-47 and MS01-83, appeared to be moderately radiosensitive.
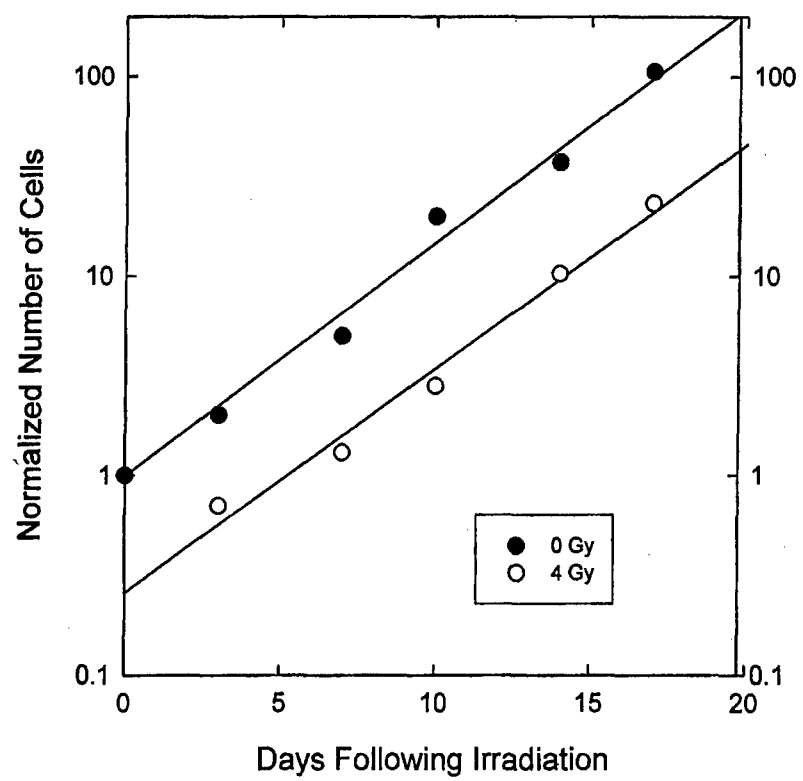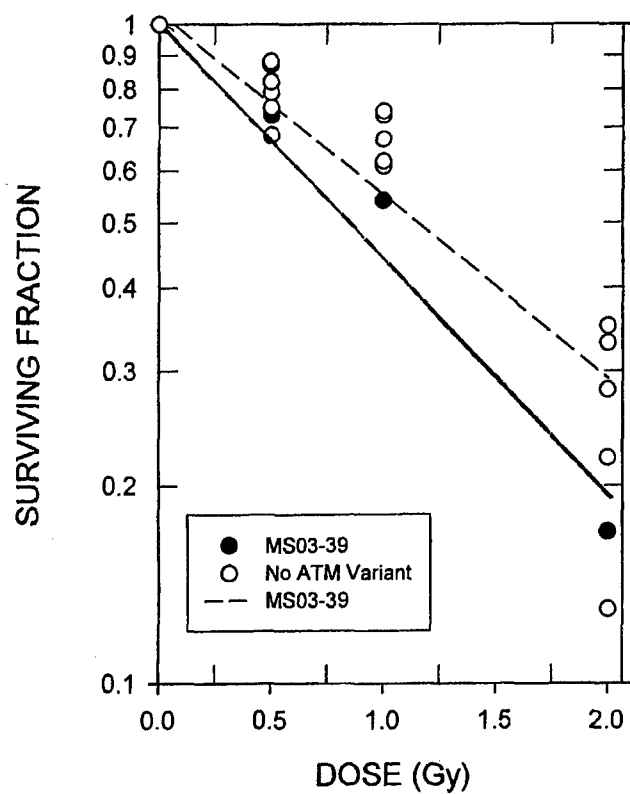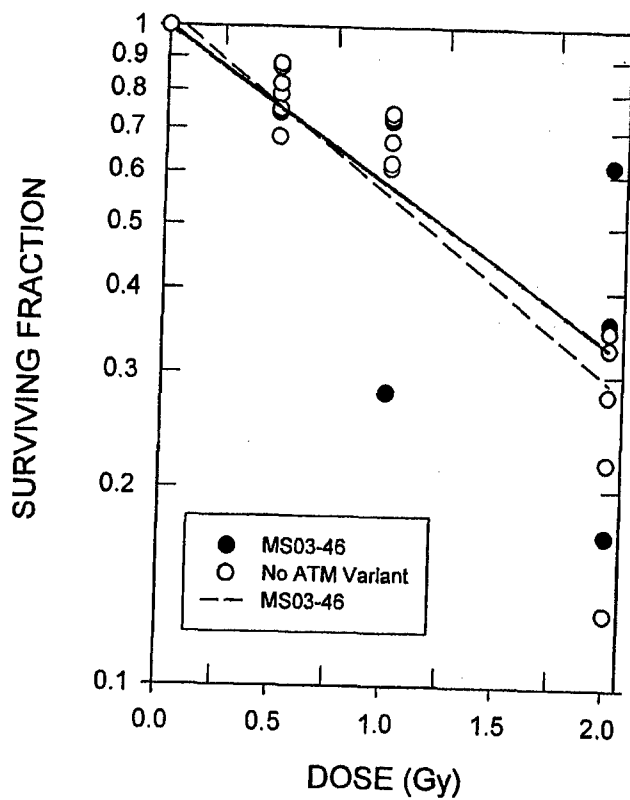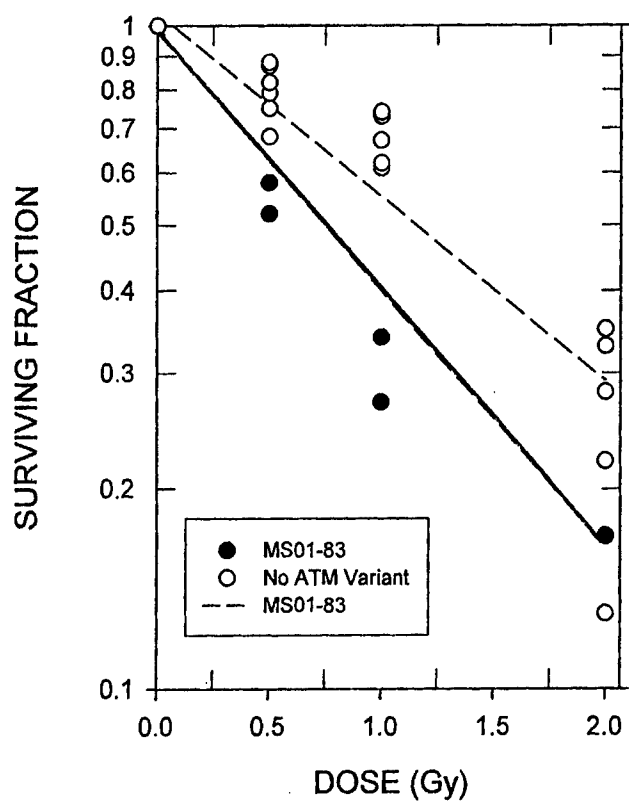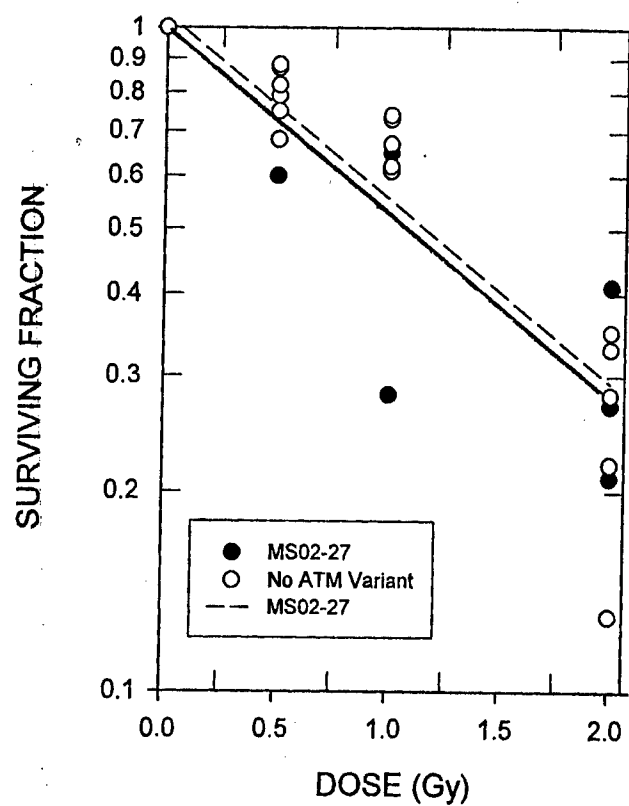
MS03-10
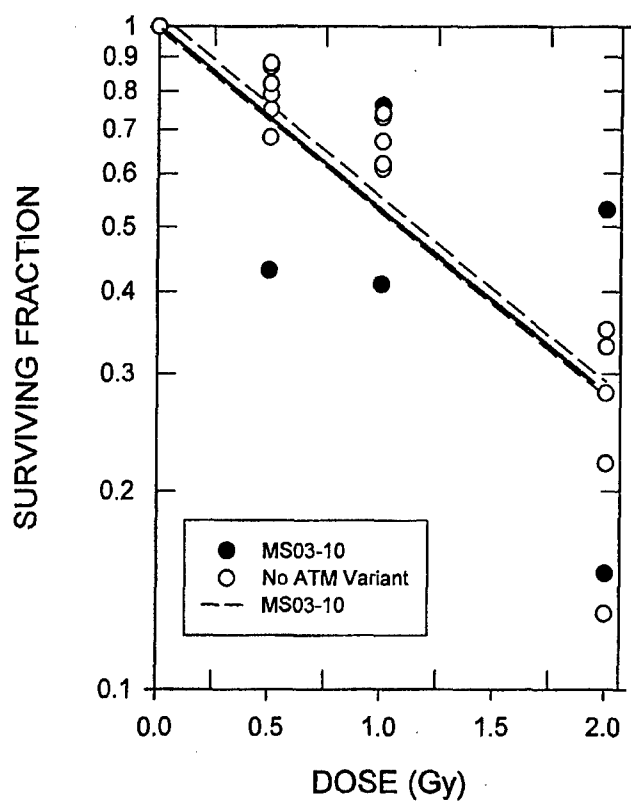
MS03-38
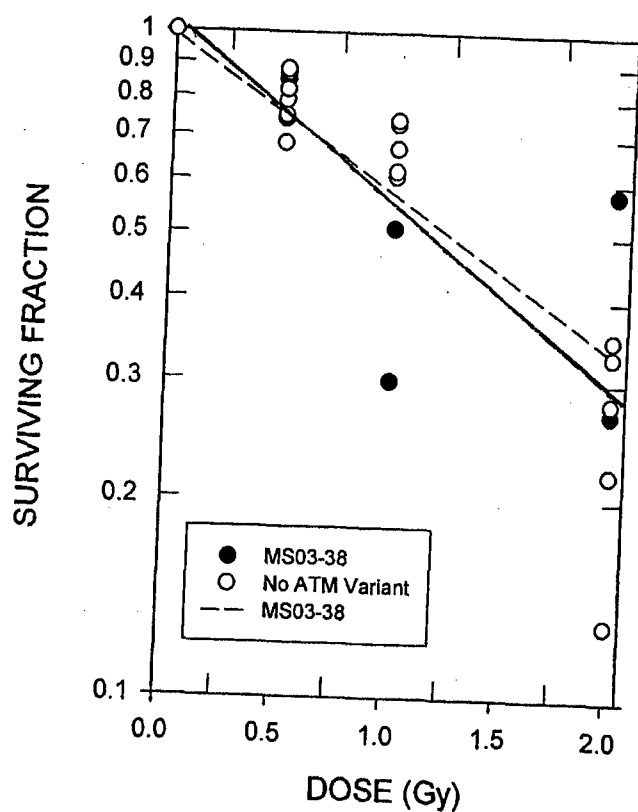
MS03-39

MS03-46

MS01-83

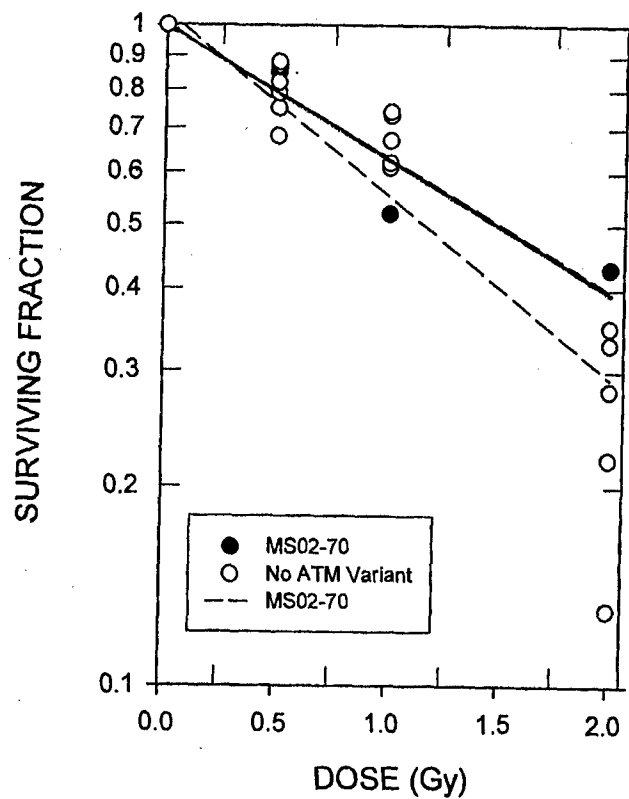
MS02-27

MS02-36
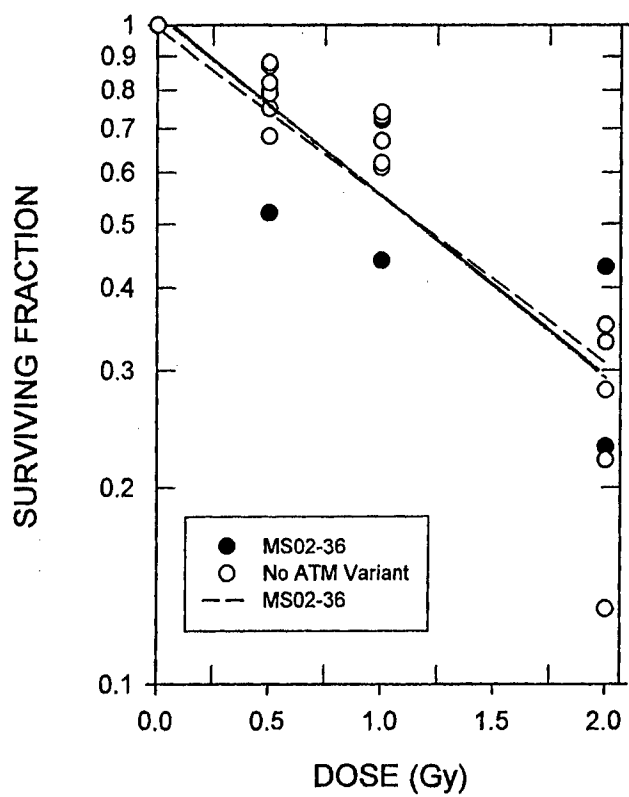
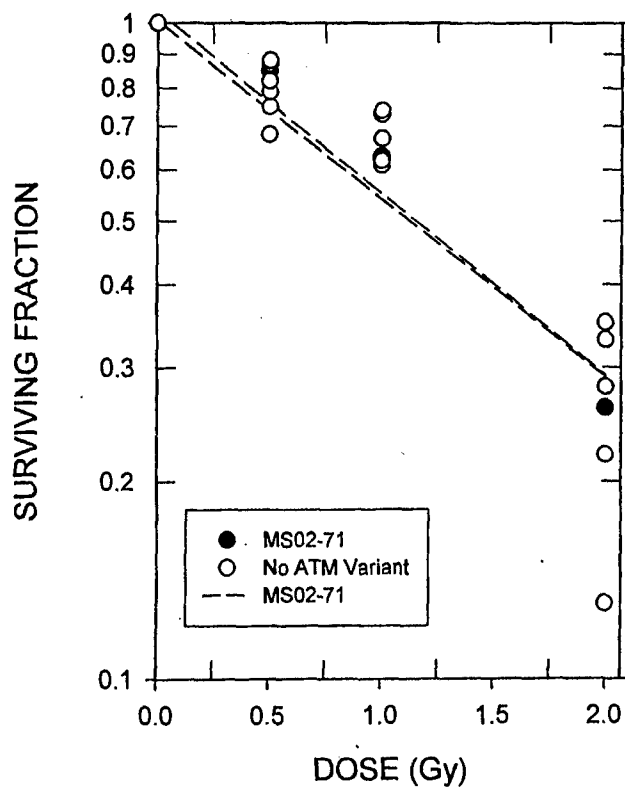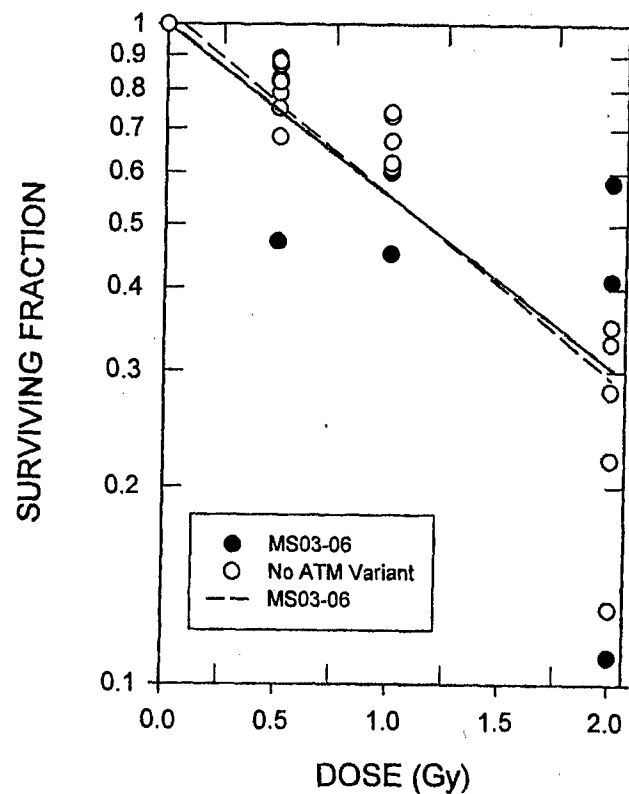

MS02-47
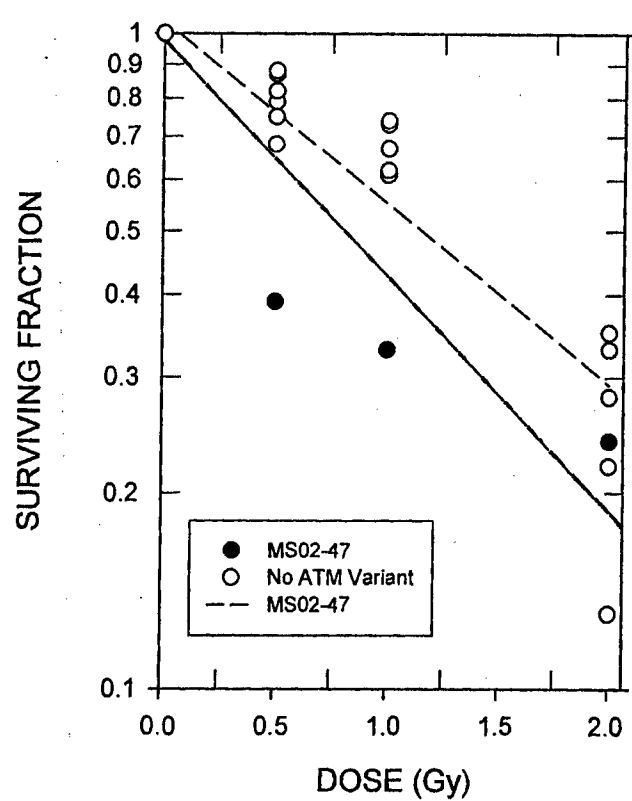
MS02-57
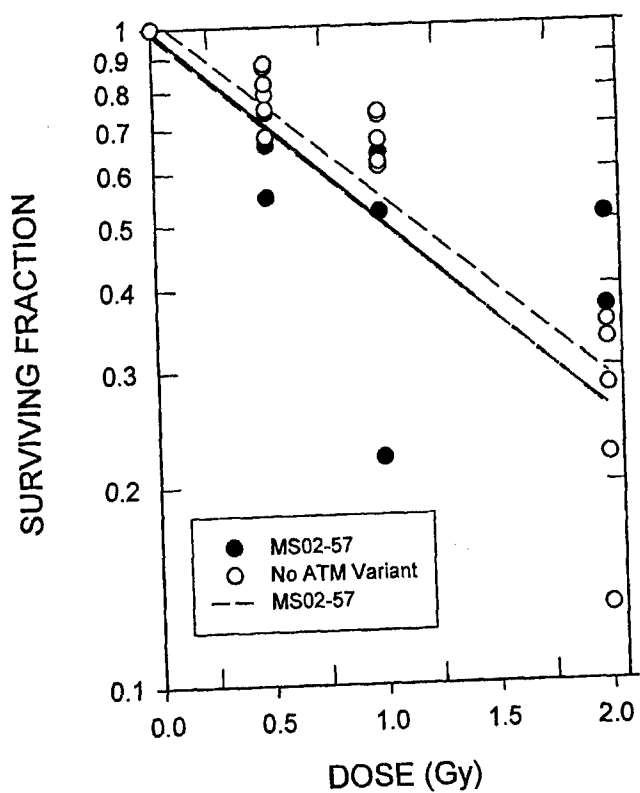
MS02-70

MS02-71

MS03-06

Key Research Accomplishments

- Western blots were performed to determine ATM protein levels and p53 phosphorylation in a total of 17 subjects not diagnosed with breast cancer. Five of these women did not possess an *ATM* variant while eight of the subjects examined harbored *ATM* variants. One of these subjects exhibited a lower level of ATM and p53 phosphorylation.

- Radiosensitivity studies were accomplished for the 5 wild type subjects and 12 women who possessed ATM variants. Cell lines derived from three of these subjects exhibited a modest level of radiosensitivity.

Reportable Outcomes

None

Conclusions

Although one subject exhibited a significantly lower level of ATM and reduced p53 phosphorylation, this did not result in cellular radiosensitivity. In general, the presence of *ATM* variants caused at most only a small increase in sensitivity to radiation for cell lines derived from women not diagnosed with breast cancer. The goal of the final year of this project will be to perform comparable functional studies with cell lines derived from women who had been diagnosed with breast cancer to determine the functional impact of *ATM* variants in these subjects. In addition, genetic screening will be completed for additional African-American breast cancer patients.

References

None

Appendices

None